Critical Reviews in Oncology/Hematology, 1993; 14:29–39
Elsevier Scientific Publishers Ireland Ltd.

ONCHEM 00040

Physical barriers to drug delivery in tumors

Brendan D. Curti

National Cancer Institute–Frederick Cancer Research and Development Center, Division of Cancer Treatment, Biological Response Modifiers Program, Clinical Research Branch, Frederick, MD 21701, USA

(Accepted 4 September 1992)

Contents

I. Introduction

In the last two decades, there have been enormous strides in our understanding of the genetic and molecular events that result in oncogenesis and the biochemical pathways that can be influenced by chemotherapy. This has translated into therapeutic successes in a number of hematologic malignancies, lymph mas and a few solid tumors such as testicular carcinoma [1–3]. However, treatment results for most solid tumors outside the adjuvant setting have been much less encouraging [4–6]. This has spurred research to define the mechanisms that cancer cells employ to resist destruction by chemotherapy agents or the immune system. Resistance mechanisms defined thus far include the p170 glycoprotein (multiple drug resistance gene product)-mediated, resistance to anthracycline and vinca alkaloid classes of chemotherapy drugs [7] and upregulation of thymidylate synthase leading to 5-fluorouracil resistance [8,9]. Tumor cells may also evade immune surveillance by expressing essentially n rmal tissue antigens rather than unique antigenic determinants that might appear foreign [10]. Strategies to vercome these defense mechanisms f neoplastic cells

Correspondence to: Dr. Brendan D. Curti, National Cancer Institute–Frederick Cancer Research and Development Center, Division of Cancer Treatment, Biological Response Modifiers Program, Clinical Research Branch, 501 West Seventh Street, Frederick, MD

trials [7,11–13]. Despite the rational basis for these studies, which has been derived mainly from in vitro experiments, the success of these approaches in patients has been limited. It is certainly possible that cancer cells possess many as yet undefined additional molecular mechanisms to defeat chemotherapy treatment strategies. If this is true, designing effective chemotherapeutic or immunologic regimens for solid tumors may prove a daunting task.

There is, however, another line of research that may prove equally important in understanding how cancer cells resist cytotoxic agents or biologics. The study of the physiology and the interstitial space of tumors, pioneered by Gullino in the 1960s and continued most recently by Jain and his co-workers [14,15], suggests a new basis for understanding at least some important mechanisms of tumor resistance. Their work has defined tumor nodules in vivo as distinct physiologic entities with unique biophysical properties compared to normal tissues. These properties cannot be deduced or reproduced by in vitro work because of the complex interaction of growing tumor cells with the new blood vessels they induce and the surrounding normal tissues. The microenvironment of tumor nodules can generate a number of physical barriers to resist a wide range of therapeutic agents. These treatment barriers cannot be ignored when attempting to design new more effective treatment regimens.

The key to understanding the unique properties of a tumor's physiology requires focus on the tumor vasculature and interstitial space. The characteristics of tumor blood vessels and their behavior in the aggregate influences the transport processes used for nutrient delivery, waste disposal and the delivery of therapeutic agents. These vessels also may also have a profound secondary effect on the properties of the interstitial space of tumors. There are a number of excellent reviews and articles that address the complicated physiology of the interstitial space [16–20]. The focus here will be to provide a general discussion of physiologic spaces, transport variables and the properties of tumor blood vessels. These basic concepts will be used to discuss selected models for the transport of therapeutic agents in tumors and measurements of the physical barriers to drug delivery in tumors.

II. Transport compartments and variables

II-A. Definition of transport spaces

In order for any therapeutic molecule to reach a neoplastic cell within a tumor nodule, several spaces with different physiologic characteristics must be traversed. For any anti-cancer agent delivered intravenously, the vascular space is the first compartment that is encountered. The molecule must then interact with the vascular endothelium, cross the endothelial membrane and tumor interstitium before eventually reaching a cancer cell. There are potential impediments to transport in each of these physiologic spaces. In order to understand these impediments, the general mechanisms for transport of any molecule must be discussed.

There are a limited number of ways that solutes (i.e., nutrients, toxins and therapeutic agents) are propelled through physiologic spaces. These are: convection, diffusion and transcytosis. The relative contribution of these variables to transport depends on the size and physical properties of the molecule and the physiologic space it is traversing (vide infra). For the purpose of this discussion, active transport across cell membranes will not be considered. Each transport variable will be discussed separately; this will be followed by an explanation of how these variables interact and which are important in tumors.

II-B. Convection

Convection is defined as the movement of solute in the direction of solvent flow. To picture this process, one can envisage a pipe in which water is flowing. If a solute, for example sodium chloride, is dissolved in the water entering the pipe, the salt molecules will travel in the direction of the flowing water. There are mathematical relationships that describe convection with defined solutes (i.e., therapeutic agents) in a given medium (i.e., the interstitial space). It is useful to examine the simplest of these relationships because it will lead to an understanding of how convection will interact with other transport variables. In a one-dimensional system, convection can be expressed as follows:

$$J_c = -CR_FK\frac{\delta p}{\delta x} \qquad (1)$$

J_c is the convective flux, C is the concentration of solute at position x, R_F is the retardation factor (the ratio of velocities of the solute and solvent; if they both move at the same speed then $R_F = 1$), K is the hydraulic conductivity of the tissue for a given solvent (a constant which depends on the solvent's viscosity) and $\delta P/\delta x$ is the hydrostatic pressure gradient at position x [20]. We can now see that convection depends on a series of constants that describe how the solute and solvent move together, multiplied by a pressure gradient.

Convection has not been measured directly in human tumors. A fluorescent photobleaching technique has been developed to address this question and has been applied to single vessels in granulation tissue and VX2 carcinoma grown in a rabbit ear chamber model [21]. The velocity of fluid moving through the interstitium of this preparation was comparable in both granulation tissue and tumor suggesting that convective flux was the same in this model. Indirect data, using gravimetric or implanted chamber techniques, have been

from the tumor interstitium to surrounding n rmal tissues that are 10–1000 times greater than n rmal tissues. These numbers represent bulk flow and do not define the events occurring at the blood vessel-tumor interface. Data obtained from measurements of arterial and venous hematocrits in tissue-isolated tumors (i.e., tumors isolated on a vascular pedicle with one artery entering the tumor nodule and one vein leaving it) show a marked hemoconcentration from the arterial to venous circulation [22]. In models using Walker 256 and MTW9 mammary carcinomas, the hematocrit of the tumor efferent veins averaged 1.043 times greater than aortic arterial blood. The authors estimated that 4.5–10.2% of the perfusing plasma volume was lost into the tumor interstitium. This implies that plasma has left the tumor blood vessels, percolated through the tumor, was discharged into the surrounding normal tissues and absorbed by peritumoral lymphatic vessels (if the fluid remained in the tumor, the nodule would sequester the entire plasma volume of the animal in a very brief time!). Alth ugh the data are suggestive of a net convective flux away from the tumor, human data do not exist for this variable.

II-C. Diffusion

Diffusion is defined as the movement of solute along a concentration gradient. Diffusion can be illustrated when a drop of ink is placed in a beaker of water. The eventual spreading of the ink throughout the beaker is dictated by diffusion. Again, taking the simplest case of a one-dimensional system, diffusion can be expressed as f llows:

$$J_D = -D \frac{\delta C}{\delta x} \qquad (2)$$

J_D is the diffusive flux, D is the diffusion coefficient of the medium and $\delta C/\delta x$ is the concentration gradient of solvent at position x [20]. This relationship tells us that diffusion is a constant that is defined by the transport medium, multiplied by a concentration gradient.

Diffusion across individual tumor blood vessels has been measured in a rabbit ear chamber model using fluorescein-labeled immunoglobulin [23]. These experiments have shown that tumor blood vessels have high effective interstitial diffusion coefficients compared to normal tissues. The high diffusion coefficient implies that transport by diffusion was greater across tumor blood vessels than normal vessels in this model. Diffusion, however, varied greatly among tumor blood vessels. Some tumor vessels have diffusion coefficients that are nearly normal, while others are approximately f ur times higher than n rmal vessels. The experimental model was n t able t distinguish whether permeability was driven primarily by convection r diffusion. Again, no direct measurements of diffusion coefficients have been made in human tumor vessels for immun gl bulin r chemotherapeutic agents.

II-D. Transcytosis

Transcytosis occurs when vascular endothelial cells form vesicles that entrap molecules or particles from the luminal surface. These vesicles are transported to the basal portion of the cell and are exocytosed. The distribution of endothelial cells that can conduct transcytosis varies among different tissue types and from the arterial to venous end of capillaries [24]. It is also thought that vesicles can coalesce to form transcapillary channels. These structures may be short-lived or permanent. Given the heterogeneous and chaotic array of tumor blood vessels (vide infra), transcytosis is thought to be insignificant compared to convection and diffusion for anti-cancer agents (with the possible exception of liposomal-encapsulated drugs) [25] and will not be included in any of the transport models discussed.

III. Interstitial space

Before discussing the interactions and relative importance of these transport variables in tumors, the composition of the interstitial space must be discussed as well as an additional physiologic variable, namely, interstitial pressure.

III-A. Composition of the interstitial space

The interstitial space of tumors is greatly different from that found in normal tissues. Furthermore, the interstitium also varies between different tumor types, sites of metastasis and perhaps even between different nodules of the same histology [26,27]. There are, however, some generalizations that can be made to illustrate the differences between normal and tumor tissues.

There is a range of values in the literature for different components of the interstitial space measured in different tumor models. The volume of the interstitial space of several animal tumor models has been measured and was found to be 36–53% of the total tumor volume [28]. this compares with 14–34% in normal tissues [29]. The total protein content of the interstitial fluid can be either higher or lower than n rmal serum, depending on the model studied. Gullino using an implanted chamber technique in a rat model showed that total protein in the interstitial fluid in a variety of tum r implants was consistently lower that aortic serum by approximately 33% [15]. Sylven and Bois compared tumor interstitial fluid sampled by micropipettes to peritoneal fluid in a variety of carcinomas grown sub-

the protein content of the tumor fluid was 5% higher than peritoneal samples. The composition of the protein matrix in tumors is also different from normal tissues. The amount of type IV collagen and glycosaminoglycans (GAG) is much greater in tumors than in the normal interstitial space [27,31]. The amino acid, lipid phosphorous and cholesterol levels also vary but are generally lower in tumors than normal serum. Direct measures of the interstitial space pH and oxygen tension in human tumors have been made. Measurements of pH in a series of melanomas ranged from 6.4 to 7.3 [32,33]. Some authors have reported tumor pH values less than 6.0 in squamous cell carcinomas and astrocytomas [32]. Direct measurements in human breast cancers have shown partial pressures of oxygen as low as 0 mmHg [34,35]. Six out of 15 nodules tested had oxygen tensions between 0–2.5 mmHg. Intertumoral oxygen tension differences were more pronounced than intratumoral measurements.

One can conclude from these data that the interstitial space of tumors is profoundly different from normal tissues. These differences represent a mechanism for potential drug resistance if the therapeutic agent is not stable or functional in an acidic or hypoxic environment. This suggests that a part of the rationale for the design of anti-cancer agents should include the development of drugs which function best in hypoxic or acidic conditions. This would seem to be easier than changing the microenvironment of established tumors.

III-B. Interstitial pressure

Interstitial pressure (IP) is defined as the sum of the hydrostatic and oncotic pressures extant in the interstitial space in the tissue of interest. There are a number of methods to measure this variable in vivo, including implantable capsules [36] and the wick-in-needle technique [37]. The data for IP in normal subcutaneous tissue and muscle tissues show a range of values in the literature from 2 to 4 mmHg [38,39]. Although IP was first noted to be elevated in animal tumors in 1950 by Young and his colleagues [40], only recently have a number of studies in humans been published. These data indicate that many human tumor nodules have greatly elevated interstitial pressures. A published series of 12 superficial metastatic melanoma deposits showed a mean IP of 14.3 and a maximum of 41 mmHg [41]. A study of epidermoid carcinomas of the head and neck also showed elevated interstitial pressures (mean 14.6, range 4 to 33 mmHg). Increased tumor volume in this study correlated directly with the interstitial pressure [42]. In a series of cervical carcinomas, there was also a correlation between IP and the oxygenation status of the tumor (i.e, the higher the IP, the lower the partial pressure of oxygen in the tumor). In this study, high interstitial pressures correlated inversely with the response to radiotherapy of these lesions [43]. The authors suggested that the hypoxia in the high IP lesions was a factor in the radioresistance of these lesions.

The reason for elevated interstitial pressures in tumors remains completely speculative. Proposed mechanisms include the absence of lymphatics in tumors and external compression of tumor blood vessels by the expanding tumor mass [20]. A recent experiment published by Lee et al, showed that nicotinamide was able to acutely decrease interstitial pressure in C3H mice bearing subcutaneous FSaII tumors [45]. This observation suggests that increases in local tumor blood flow or systemic blood pressure changes mediated by nicotinamide may also be important in regulating interstitial pressures. Tumor water content in this study also correlated with IP and may contribute to its pathogenesis. Hyperthermia in an animal model was also found to decrease IP and improve therapeutic response as measured by growth delay of the tumors compared to controls [46]. The impact of hyperthermia on IP may have been through damage to small tumor blood vessels [47]. Any mechanism proposed must account not only for the elevated pressures discussed above but also for the observation that IP is transmitted uniformly through tumor nodules. IP measured by a micropuncture technique in mammary carcinomas grown as tissue-isolated tumors in rats was essentially uniform throughout the tumor nodules studied. It should be noted that there was a sharp pressure gradient in the outermost 0.5 mm of the tumor nodules, extending into the surrounding normal tissues [48]. Although the data remain preliminary and the mechanism unclear, it is reasonable to conclude that IP is elevated in many tumor nodules compared to normal tissues. With this background, one can now ask how IP might impact on diffusion and convection and what implications this may have for drug delivery.

III-C. Interaction of transport variables

We have seen thus far that tumor blood vessels can have high effective diffusion coefficients and that diffusion and convection are quite different in tumors compared to normal tissues. The interaction of these variables will now be examined and the importance of each to the delivery of anti-cancer drugs in tumors will be discussed.

To accomplish this goal, one can look at the ratio of diffusion to convection for molecules of different sizes traveling through an interstitial space with differing GAG contents. It should be noted that of all the protein and carbohydrate constituents of the interstitial space, GAG's have the most impact on these transport variables [49]. The ratio of diffusion to convection is defined as follows:

$$\lambda = \frac{\text{Diffusive flux}}{\text{Convective flux}} = \frac{D\,\Delta C}{\quad} \qquad (3)$$

This expression was obtained by dividing (1)/(2) above [49]. $\Delta C/C$ and Δp have been measured in animal models. Their values are 1.0 (dimensionless) and 30 mmHg, respectively. Incorporating these into equation 3 yields the following approximation:

$$\lambda = 2.5 \cdot 10^{-5}\ D/R_f K \qquad (4)$$

Equation 4 can be used to depict the relationship between the molecular mass of a therapeutic agent and GAG content of the interstitium for different ratios of convection to diffusion and different reflection coefficients. One of the implications of this equation is that larger molecules (i.e., those with molecular masses greater than approximately 800 Da) move much more readily by convection whereas smaller molecules move easily by diffusion alone. To illustrate the potential importance of these transport variables, one can ask how long it would take an immunoglobulin (IgG) molecule to travel one centimeter by diffusion alone. The answer is approximately 7 months [44]! Convection, therefore is extremely important to the transport of large therapeutic molecules. We can now try to answer whether convection or diffusion predominates in the interstitial space of tumors.

As discussed above, direct and indirect evidence suggests that diffusion and convection are abnormal in tumors. To try and determine which one predominates in tumors, we will focus on what happens at the interface between blood vessels and the interstitium of tumors. Here, the blood vessels of interest are those that participate in the exchange of nutrients or therapeutic agents. These vessels are thought to be post-capillary venules. Since convection is the rate-limiting process for large molecules, it will be examined first. The relation for convective flux (JC_c) across a blood vessel wall is given by Starling's Law [50]:

$$J_c = L_p S[(p_v - p_i) - \sigma_T(\pi_v - \pi_i)] \qquad (5)$$

L_p is the hydraulic conductivity of the blood vessel wall, S is the surface area of the vessels, p_v and p_i are the vascular and interstitial pressures, respectively, σ_T is the osmotic reflection coefficient (this relates the movement of solute under conditions of high filtration rate and n concentration difference) and π_v and π_i are the smotic pressures of the intravascular plasma and the interstitial fluid, respectively.

Dvorak and Clauss [51–54] am ng others have sh wn that tumor vessels are extremely leaky to large protein molecules, which may be mediated by specific tumor vessel permeability factors. Furthermore, assays of tumor interstitial fluid have shown directly that there is a high protein content [30]. This would render the quantity $(\pi_v - \pi_i)$ equal to or approximately zero, since proteins would distribute equally [illegible]

data for p_v in tumor blood vessels; however in an implanted chamber model using a mammary adenocarcinoma [55], both tumor arteriolar and venous capillary pressures were similar to normal control vessels. Assuming that the published values for p_i (approximately 15–40 mmHg) in humans are representative of most tumor nodules and that the value for p_v in tumors is close to the normal value (0–5 mmHg), then the quantity $(p_v - p_i)$ is negative. This implies that there is no convection across nutrient exchange vessels in tumors. Diffusion, therefore, becomes the only mode of transport available to therapeutic agents at the level of the vasculature most important to the delivery of any therapeutic agent. As we have seen, diffusion is a good way to move small molecules (molecular weight less than 800 Da) rapidly through the tumor interstitium, but an extremely poor way to move larger molecules through this space over a short period of time.

The physiologic argument presented above is limited by the fact that all of the equations are approximations that account for only a limited number of the possible important variables. Additional physiologic data are needed to fully validate these relations in tumors. Despite these shortcomings, a significant physiologic impediment to the delivery of therapeutic agents, which is particularly relevant to high molecular weight molecules such as biologic response modifiers and immunoglobulins, is strongly suggested by consideration of these variables. We will now examine additional mechanisms that may inhibit the delivery of therapeutic agents that stem from the unique geometry and flow characteristics of tumor blood vessels.

III-D. Tumor blood vessels

The process of angiogenesis in normal tissues and tumors has been the subject of intensive research in recent years [56–58]. There have also been a number of studies examining the microscopic characteristics of tumor blood vessels and their overall organization [59,60]. Much less is known about the aggregate flow characteristics of these vessels and how they interact with surrounding normal vessels. These studies are difficult because of the extreme variability of tumor vessels. The variation encompasses not only the number of blood vessels (ranging from 0.8 to 25% of the total tumor volume in the same animal model [61]) but their characteristics over different tumor histologies, different nodules of the same histology and different regions within the same nodule as well. Despite these difficulties, it is possible to make some valid generalizations about features that may impact on the transport of drugs to tumors.

III-E. Tumor vessel characteristics

brane and surrounding pericytes [62]. The branching pattern of normal vessels as they progress from large arteries to arterioles, capillaries, post-capillary venules and veins is orderly and can be described with remarkable precision by using a mathematical tool known as fractal geometry [63]. Certain specialized blood vessels, such as those found in the glomerulus or those that make up the blood-brain barrier have additional unique anatomic and functional characteristics [64–66], yet they have the same basic framework outlined above. Normal vessels dilate and contract in a stereotypic way when subjected to a variety of pharmacologic agents [67,68].

Despite the fact that tumor blood vessels are thought to be recruited from surrounding normal host vessels, they do not have a consistent structural motif. The endothelial lining of tumor vessels is patchy and may include tumor cells in direct contact with flowing blood. The basement membrane is also irregular in distribution and thickness and has a different composition compared to normal vessels [69,70]. There are no pericytes or myocytes associated with tumor vessels [51]. This may account for the fact that tumor vessels have a much diminished response to a variety of vasoactive drugs [71].

III-F. Tumor vessel geometry

There are a number of unique blood vessel configurations present in tumors but not in most normal tissues. An elegant characterization of these geometries has recently been published by Less and colleagues [59], who systematically described the branching pattern and vessel diameters from vascular casts of mammary carcinomas grown as tissue-isolated tumors in rats. These authors noted the presence of vessel true loops (single vessels taking a 360 degree circular path before branching), self loops (vessels bifurcating and then rejoining without additional branches), trifurcations and the direct branching of moderate sized arterioles into capillary networks. They did not observe arterial-venous shunts; however, other authors have observed these structures [72,73]. Vessel diameters and lengths were also measured in this model, showing that tumor vessel diameters were greater and lengths shorter for a given vessel generation number than published values for normal tissues (the generation number denotes the number of branchings that have occurred from the main arterial vessel entering the tumor).

In addition, the overall organization of tumor blood vessels was noted to be unique. Some nodules displayed central vascularization with large vessels occupying the interior portion of the nodule. Other vascular casts showed a pattern of peripheral organization, with the largest number of vessels covering the surface of the nodule. With either of these vascular schemes, the distance between blood vessels and tumor cells could be quite large (in some areas, approaching 1 cm).

These regional differences and the uniqueness of tumor vessel organization illustrate an important and recurring theme in tumor physiology, namely, that tumors nodules are not alike. Even if an understanding of the dynamics of drug delivery in the setting of peculiar blood vessel geometries is achieved, the relative contribution of each geographic region of the tumor would have to be assessed individually to arrive at an accurate determination of blood flow in a particular nodule.

III-G. Flow characteristics in tumor vasculature

A number of experimental methods have been used to study tumor blood flow including laser Doppler [74], radioactive tracer washout [75] and perfusion techniques [76]. Blood flow in tumors can vary significantly, even among tumors of the same size derived from the same cell line [77]. Despite this variability, a number of researchers have concluded that as tumor size increases, total perfusion decreases [78]. This has been measured directly by ^{133}Xe clearance and is also manifest indirectly by observing the geometric resistance of tumor blood vessels, which increases with tumor weight. The geometric resistance of tumor vessels is one to two orders of magnitude higher than normal vessels. Using positron emission tomography and inhaled ^{15}O as a tracer, Wilson and colleagues have recently made direct measurements of total blood flow in human breast tumors [79]. The ^{15}O is converted by carbonic anhydrase in the lung to $H_2{}^{15}O$, which distributes into arterial blood. They determined that the mean blood flow in normal breast tissue was 5.6 ml/dl/min, compared to 29.8 ml/dl/min in breast tumors. There was no correlation between tumor size or prognosis and tumor blood flow in these patients. The degree of tumor necrosis or vascularity was not examined in this study.

Tumor blood flow can be altered by a number of factors. Viscosity of blood in tumors has been measured and was found to be greater than normal vessels. Viscosity also varies directly with hematocrit and increases with decreasing blood pressure [80]. The reason that intratumoral blood viscosity is different than normal vessels is thought to stem from two causes. Extravasation of plasma from leaky vessels (vide supra) causes a relative hemoconcentration. Also, red blood cells normally stream in the center of blood vessels as long as the vessel diameter is greater than one red cell diameter. This creates a cell-free marginal layer at the vessel wall, which results in decreased blood viscosity. This is known as the Fahraeus-Lindqvist effect. This phenomenon is decreased in tumors [78] secondary to irregular vessels. This ultimately results in non-linear flow, increased turbulence, increased rouleaux formation and elevated blood viscosity. Cytokines can also alter tumor blood flow. This was documented by Kluge,

et al. [81], who showed that tumor necrosis factor α and lymphotoxin lower tumor blood flow and increase tumor vascular resistance. This resulted in a larger decrement in tumor energy consumption as measured by ^{31}P-nuclear magnetic resonance spectroscopy. Hydralazine and some anesthetic agents was shown by other investigators [82] to also decrease tumor blood flow in a mouse model. These investigators found no effect of hydralazine on two human xenografts in nude mice.

IV. Transport models in tumors

There are scant data defining the amount of a chemotherapy or immunotherapy drug that reaches tumor nodules in humans. Most of the extant information comes from models examining the amount of a given monoclonal antibody localizing to tumors or cellular trafficking to human tumor nodules after adoptive immunotherapy. In general, the monoclonal antibodies studied for therapy or their use in imaging human tumors after intravenous administration will localize to the tumor, but will be unevenly distributed [83–85]. Common distribution patterns include finding the antibody in the peritumoral area or focally deposited around intratumoral blood vessels. The amount of antibody measured in the tumor is also much less than predicted by in vitro binding experiments. This is illustrated by a study by Shockley et al, who examined a melanoma xenograft model. They found that melanoma-specific antigen concentrations were 15–70 times less than that suggested by a three compartment kinetic model, which translated into markedly lower antibody concentrations in the tumor 72 h after injection [86]. Another study compared diphtheria toxin and an immunoconjugate of diphtheria toxin and the human transferrin receptor in a human xenograft tumor model [87]. Although the plasma-to-tissue transport constants were high in the tumors, the amount of immunotoxin that localized in vivo was 530 times less than predicted by in vitro binding affinities. These findings suggest that lower expression of antigen binding sites and decreased accessibility of the antibody in vivo present significant barriers to antibody treatment. Similarly, adoptively transferred cells such as lymphokine-activated killer cells (LAK) are not generally found in tumor sites [88]. Tumor infiltrating lymphocytes (TIL), which are phenotypically different from LAK are reported to reproducibly reach their tumor targets [89]. The reasons for this remain to be elucidated. Strategies to improve antibody localization by enhancing blood vessel leakiness are also being pursued. Interleukin-2 and monoclonal antibodies conjugated to interleukin-2 have demonstrated in animal models [90,91] that increased concentrations of monoclonal antibodies in tumors are attainable by this approach.

The dynamics of cell movement are likely to be more complicated than the forces that govern monoclonal antibodies or chemotherapy drug movement since cells can expend energy to move along chemotactic gradients and have additional interactions with the vasculature mediated by adhesion molecules [92]. Nevertheless, common forces such as diffusion, convection, interstitial pressure and binding site barriers affect both. There have been a number of recently developed models that account for some of these variables, which will be discussed below.

In the development of monoclonal antibodies, two of the central issues are the binding affinity of the antibody and the distribution of antigen within the tumor mass. Fujimori and colleagues [93,94] developed a spherical tumor nodule model that looked at the distribution of antibodies with varying affinities and different tumor antigen concentrations. Their model also took into account the effective interstitial diffusion coefficient, capillary permeation, the initial concentration of the antibody in the serum and the valence of the antibody. This modelling scheme showed that as the number of antibody-antigen binding events increased, or the affinity of the antibody increased, the percolation of the antibody through the tumor decreased. This reduced the heterogeneity of antibody distribution and produced a binding site barrier.

Sung et al, developed a plasma and tissue compartmental model that accounted for interstitial fluid flow and employed the Langmuir isotherm to estimate antibody-antigen binding [95]. Using this approach, the model predicted that increasing antibody affinity at low doses of antibody would result in increased tumor uptake. When antigen saturation was approached, binding affinity had a smaller effect. Another implication of this model was that if tumor antigen density could be increased, antibody localization could also be increased. This point was illustrated by an animal model comparing two different melanomas with different antigen concentrations grown subcutaneously. The antibodies used had similar affinities for antigen in the tumors studied. The melanoma with the greater antigen density (SK-MEL-2) had greater antibody uptake. Other researchers [96–98] have also concluded that antigen density is of salient importance to antibody distribution in model systems that used antibodies to epidermal growth factor receptors, carcino-embryonic antigen and cell-surface ovarian carcinoma antigens.

Another modelling approach incorporated interstitial pressure as a variable to explain heterogeneous antibody distribution in tumors. In the model developed by Jain and Baxter [43], IP opposed the tendency of fluid and macromolecules to leave tumor blood vessels. It also resulted in net convective forces that are directed radially outward and are of a magnitude sufficient to counteract the tendency of any molecule present in the

the substance f the tumor. A number of assumptions were made, including that the tumor was uniformly perfused, IP was spatially dependent and that the macromolecules modelled were free to move in the tumor interstitium (i.e., no binding in the tumor). With these constraints, the model predicted that smaller molecules, such as Fab fragments reached higher concentrations for a given radial position in the tumor than IgG after bolus injections. Continuous infusion of the antibody resulted in higher intratumoral concentrations of both IgG and Fab. Furthermore, the distribution of IgG, $F(ab)_2$ or Fab predicted by the model was heterogeneous. Thus, by modelling a physiologic variable and not the binding characteristics of the therapeutic agent, it was concluded that tumors would have regions with different concentrations of macromolecules, delivered intravenously.

V. Conclusions

The chaotic nature of tumor blood vessels and blood flow, the varied composition of the tumor interstitium and disturbed convection and diffusion in the interstitial space of tumors all create significant potential physical barriers to the delivery of therapeutic agents to neoplastic cells in vivo.

This hostile microenvironment is not duplicated or predicted by any in vitro system and is only partially accounted for by extant computer models. Similarly, animal models employing very small tumor nodules that possess a nascent or absent vascular network do not mimic the physiology discussed here. If the aggregate physiologic behavior of tumor nodules is not taken into account, then any therapy, no matter how rationally designed or effective in vitro, is likely to be diminished in patients with large tumor burdens.

Most of the physiologic data discussed here were developed in animal systems, which though instructive, do not definitively address the complicated dynamics of the interstitial space. The physiologic models and relationships for convection and diffusion are also crude approximations of a changing and heterogeneous tumor microenvironment. A great deal more needs to be understood about tumor blood vessels and the interstitium of human tumors. Recent advances in imaging technologies such as NMR and PET scanning may provide additional insights about how cancer therapies interact with their tumor targets in vivo. One of these modalities (NMR spectroscopy) has recently been used to estimate the amount of a chemotherapy agent (5-fluorouracil) in a number of liver metastases from colon carcinoma in humans [99]. The responses f the tumor correlated with the am unt f 5-fluorouracil trapped in the tumor. Although n ne of the physiologic variables discussed above was directly measured by this technique, the question asked by this research is central to this discussion, namely, does the treatment get to the tumor?

Knowledge about the physical barriers to drug delivery in tumors is a work in progress. Overcoming these barriers may prove to be just as important as thwarting the molecular mechanisms of tumor resistance that have been elucidated in recent years. Studies of the tumor vasculature and interstitium will answer at least one very important question in cancer therapeutics, namely, does the drug reach its target and stay there. It is only when this is understood that the full potential of chemotherapy, gene therapy and immunotherapy can be realized.

Acknowledgments

The author is greatly appreciative of the thoughtful criticisms made by Dr. Dan Longo and Dr. Walter Urba. Also, the author would like to thank Sharon Lewis for her expert help in the preparation f the manuscript.

Biography

Brendan D. Curti received an M.D. degree from the Georgetown University School of Medicine. He is currently a Senior Staff Fellow at the National Cancer Institute and a Clinical Assistant Professor of Medicine at Georgetown University.

Reviewer

This paper was reviewed by Cynthia Sung, Ph.D., National Institute of Health, BEIP/NCRP, Bethesda, MD 20892, USA.

References

1 Longo DL, DeVita Jr VT, Duffey PL et al. Superiority of proMACE-cytaBOM over proMACE-MOPP in the treatment of advanced diffuse aggressive lymphoma: results of a prospective randomized trial. J Clin Oncol 9:25–38, 1991.

2 Urba WJ, Longo DL. Hodgkin's disease. N Engl J Med 326: 678–688, 1992.

3 Einhorn LH. Treatment of testicular cancer: a new and improved model. J Clin Oncol 8:1777–1781, 1990.

4 Morton RF, Jett JR, McGinnis WL et al. Thoracic radiation therapy alone compared with combined chemoradiotherapy for locally unresectable non-small cell lung cancer. Ann. Intern Med 115:681–686, 1991.

5 Muss HB, Case LD, Richards F et al. Interrupted versus continuous chemotherapy in patients with metastatic breast cancer. N Engl J Med 325:1342–1348, 1991.

6 Gittes RF. Carcinoma of the Prostate. N Engl J Med 324:236–245, 1991.

7 Salmon SE, Dalton WS, Grogan TM et al. Multidrug-resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. Blood 78:44–50, 1991.

8 Peters GJ, van Groeningen CJ. Clinical relevance of biochemical modulation of 5-fluorouracil. Ann Oncol 2:469–480, 1991.

9 Zhang ZG, Harstrick A, Rustum YM. Mechanisms of resistance t fluoropyrimidines. Semin Oncol 19:4–9, 1992.

10 Herlyn M, Menrad A, Koprowsky H. Structure, function and clinical significance of human tumor antigens. J Natl Cancer Inst 82:1883–1889, 1990.

11 Dalton WS, Grogan TM, Meltzer PS et al. Drug resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J Clin Oncol 7:415–424, 1989.

12 Gottesman MM, Pastan I. Clinical trials of agents that reverse multidrug-resistance. J Clin Oncol 7:409–411, 1989.

13 Levin VA, Prados MD. Treatment of recurrent gliomas and metastatic brain tumors with polydrug protocol designed to combat nitrosourea resistance. J Clin Oncol 10:766–771, 1992.

14 Jain RK. Delivery of novel therapeutic agents in tumors: Physiological barriers and strategies. J Natl Cancer Inst 81:570–576, 1989.

15 Gullino PM, Clark SH, Grantham FH. The interstitial fluid of solid tumors. Cancer Res 24:780–797, 1964.

16 Baxter, LT, Jain, RK. Transport of fluid and macromolecules in tumors I. Role of interstitial pressure and convection. Microvasc Res 37:77–104, 1989.

17 Baxter LT, Jain RK. Transport of fluid of macromolecules in tumors II. Role of heterogeneous perfusion and lymphatics. Microvasc Res 40:246–263, 1990.

18 Baxter LT, Jain RK. Transport of fluid and macromolecules in tumors III. Role of binding and metabolism. Microvasc Res 41:5–23, 1991.

19 Baxter LT, Jain RK. Transport of fluid and macromolecules in tumors IV. A microscopic model of the perivascular distribution. Microvasc Res 41:252–272, 1991.

20 Jain, RK. Transport of molecules in the interstitium: a review. Cancer Res 47:3039–3051, 1987.

21 Chary, SR, Jain, RK. Direct measurement of interstitial convection and diffusion of albumin in normal and neoplastic tissues by fluorescence photobleaching. Proc Natl Acad Sci 86:5385–5389, 1989.

22 Butler TP, Grantham FH, Gullino PM. Bulk transfer of fluid in the interstitial compartment of mammary tumors. Cancer Res 35:3084–3088, 1975.

23 Clauss MA, Jain RK. Interstitial transport of rabbit and sheep antibodies in normal and neoplastic tissues. Cancer Res 50:3487–3492, 1990.

24 Taylor AE, Granger DN. Exchange of macromolecules across the microcirculation. In: Renkin EM, Michel CC, eds. Handbook of Physiology-The Cardiovascular System, Section 2, Volume IV, Microcirculation, Chapter 11. American Physiology Society, Bethesda, MD 1984; 467–520.

25 Gabizon AA. Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes. Cancer Res 52:891–896, 1992.

26 Gullino PM, Grantham FH. The influence of the host and the neoplastic cell population on the collagen content of a tumor mass. Cancer Res 23:648–653, 1963.

27 Gullino PM, Grantham FH, Clark SH. The collagen content of transplanted tumors. Cancer Res 22:1031–1037, 1962.

28 Gullino PM, Grantham FH, Smith SH. The interstitial water space of tumors. Cancer Res 25:727–731, 1965.

29 O'Conner SW, Bale WF. Accessibility of circulating immunoglobulin G to the extravascular compartment of solid rat tumors. Cancer Res 44:3719–3723, 1984.

30 Sylven B, Bois I. Protein content and enzymate assays of interstitial fluid from some normal tissues and transplanted mouse tumors. Cancer Res 20:831–836, 1960.

31 Choi HU, Meyer K, Swarm R. Mucopolysaccharide and protein-polysaccharide of a transplantable rat chondrosarcoma. Proc Natl Acad Sci 68:877–879, 1971.

32 Ashby BS. pH studies in human malignant tumors. Lancet ii: 312–315, 1966.

33 Thistlewaite AJ, Leeper DB, Moylan DJ, Nerlinger RE. pH distribution in human tumors. Int J Radiat Oncol Biol Phys 11: 1647–1652, 1985.

34 Vaupel P, Schlenger K, Knoop C, Hockel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurements. Cancer Res 51:3316–3322, 1991.

35 Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply and metabolic microenvironment of human tumors: a review. Cancer Res 49:6449–6465, 1989.

36 Guyton AC. A concept of negative interstitial pressure based on pressures in implanted perforated capsules. Circ Res 12:399–414, 1963.

37 Wiig, H, Reed RK, Aukland, K. Measurement of interstitial fluid pressure: comparison of methods. Ann Biomed Eng 14:139–151, 1986.

38 McMaster PD. The pressure and interstitial resistance prevailing in the normal and edematous skin of animals and man. J Exp Med 84:473–494, 1946.

39 Hargens HR, Mubarak SJ, Owen CA, Garetto LP, Akeson, WH. Interstitial pressure in muscle and compartment syndromes in man. Microvasc Res 14:1–10, 1977.

40 Young JS, Lumsden CE, Stalker AL. The significance of the 'tissue pressure' of normal testicular and neoplastic (Brown-Pearce carcinoma) tissue in the rabbit. J Path Bacteriol 62:313–333, 1950.

41 Boucher Y, Kirkwood JM, Opacic D, Desantis M, Jain RK. Interstitial hypertension in superficial metastatic melanomas in humans. Cancer Res 51:6691–6694, 1991.

42 Gutmann R, Leunig M, Feyh J et al. Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. Cancer Res 52:1993–1995, 1992.

43 Roh HD, Boucher Y, Kalnicki S, Buchsbaum R, Bloomer WD, Jain RK. Interstitial hypertension in carcinoma of uterine cervix in patients: possible correlation with tumor oxygenation and radiation response. Cancer Res 51:6695–6698, 1991.

44 Jain RK, Baxter LT. Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure. Cancer Res 48:7022–7032, 1988.

45 Lee I, Boucher B, Jain RK. Nicotinamide can lower tumor interstitial pressure: mechanistic and therapeutic implications. Cancer Res 52:3237–3240, 1992.

46 Leunig M, Goetz AE, Dellian M, Zetterer G, Gamarra F, Jain RK, Messmer K. Interstitial fluid pressure in solid tumors following hyperthermia: possible correlation with therapeutic response. Cancer Res 52:487–490, 1992.

47 Endrich B, Hammersen F, Messmer K. Hyperthermia-induced changes in tumor microcirculation. Recent Results Cancer Res. 107:44–59, 1988.

48 Boucher Y, Baxter LT, Jain RK. Interstitial pressure gradients in tissue-isolated and subcutaneous tumors: implications for therapy. Cancer Res 50:4478–4484, 1990.

49 Swabb EA, Wei J, Gullino PM. Diffusion and convection in normal and neoplastic tissues. Cancer Res 34:2814–2822, 1974.

50 Kedem O, Katchalsky A. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. Biochim Biophys Acta 27:229–245, 1958.

51 Dvorak HF, Nagy JA, Dvorak JT, Dvorak AM. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. Am J Pathol 133:95–109, 1988.

52 Roberts WG, Hasan T. Role neovasculature and vascular permeability on the tumor retention of photodynamic agents. Cancer Res 52:924–930, 1992.

53 Brock TA, Dvorak HF, Senger DR. Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. Am J Pathol 138:213–221, 1991.

54 Kock PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 246:1309–1312, 1989.

55 Peters W, Teixeira M, Intaglietta M, Gross JF. Microcirculatory studies in rat mammary carcinoma. I. Transparent chamber method, development of microvasculature and pressures in tumor vessels. J Natl Cancer Inst 65:631–642, 1980.

56 Dvorak HF, Nagy JA, Dvorak AM. Structure of solid tumors and their vasculature: implications for therapy with monoclonal antibodies. Cancer Cells 3:77–85, 1991.

57 Blood CH, Zetter BR. Tumor interactions with the vasculature: angiogenesis and tumor metastasis. Biochim Biophys Acta 1032:89–118, 1990.

58 Grant DS, Tashiro B, Segui-Real Y, Yamada Y, Martin G, Kleinman H. Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. Cell 58:933–943, 1989.

59 Less JR, Skalak TC, Sevick EM, Jain RK. Microvascular architecture in a mammary carcinoma: branching patterns and vessel dimensions. Cancer Res 51:265–273, 1991.

60 Llombart-Bosch A, Peydro-Olaya A, Pellin A. Ultrastructure of vascular neoplasms. A transmission and scanning electron microscopical study based on 42 cases. Pathol Res Pract 174(1/2): 1–41, 1982.

61 Jain RK. Determinants of tumor blood flow, a review. Cancer Res 48:2641–2658, 1988.

62 Rodkiewicz CM. Arteries and arterial blood flow. Springer-Verlag, New York, NY 1983.

63 Mandelbrot BB. The fractal geometry of nature. W.H. Freeman and Company, New York, NY, 1983.

64 Waeber B, Burnier M, Nussberger J, Brunner HR. Role of atrial natriuretic peptides and neuropeptide Y in blood pressure regulation. Horm Res 34:161–165, 1990.

65 Schunkert H, Ingelfinger JR and Dzau VJ. Evolving concepts of the intrarenal renin-angiotensin system in health and disease: contributions of molecular biology. Renal physiol biochem 14:146–154, 1990.

66 Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 323:27–36, 1990.

67 Williams GH. Converting-enzyme inhibitors in the treatment of hypertension. New Engl J Med 319 (23):1517–1525, 1988.

68 Dean CR, Mailing T, Dargie HJ, Reid JL, Dollery CT. Effect of propranolol on plasma norepinephrine during sodium nitroprusside-induced hypotension. Clin Pharmacol Ther 27:156–163, 1980.

69 Kaiser HE. Stroma, generally a non-neoplastic structure of the tumor. Cancer Growth Prog 3:1–8, 1989.

70 Ingerber DE, Folkman J. Extracellular matrix, endothelial cell shape modulation and control of angiogenesis: potential targets for antitumor differentiation therapy. Serono Symp Publ Raven Press 45:111–124, 1988.

71 Thomas C, Counsell C, Wood P, Adams GE. Use of fluorine-19 nuclear magnetic resonance spectroscopy and hydralazine for measuring dynamic changes in blood perfusion volume in tumors in mice. J Natl Cancer Inst 84:174–180, 1992.

72 Warren BA. The vascular morphology of tumors. In: Peterson, HI, ed. Tumor Blood Circulation: Angiogenesis, Vascular Morphology and Blood Flow of Experimental and Human Tumors. CRC Press, Boca Raton, FL, 1979.

73 Gothlin J. Arteriovenous shunting in carcinomas evaluated by a dye dilution technique. Scand J Urol Nephrol 11:159, 1977.

74 Vaupel P, Kluge M, Ambroz C. Laser doppler flowmetry in subepidermal tumors and in normal skin of rats during localized ultrasound hyperthermia. Int J Hyperthermia 4:307–321, 1988.

75 Mantyla M, Heik nen J, Perkki J. Regi nal blood flow in human tumors measured with argon, krypton and xenon. Br J Radiol 61:379, 1988.

76 Tveit E, Weiss L, Lundstam S, Hultborn R. Perfusion characteristics and norepinephrine reactivity of human renal carcinoma. Cancer Res 47:4709–4713, 1987.

77 Lyng H, Skretting A, Rofstad EK. Blood flow in six human melanoma xenograft lines with different growth characteristics. Cancer Res 52:584–592, 1992.

78 Sevick EM, Jain RK. Geometric resistance to blood flow in solid tumors perfused ex vivo: effects of tumor size and perfusion pressure. Cancer Res 49:3506–3512, 1989.

79 Wilson CB, Lammertsma AA, McKenzie CG, Sikora K, Jones T. Measurements of blood flow and exchanging water space in breast tumors using positron emission tomography: a rapid and noninvasive dynamic method. Cancer Res 52:1592–1597, 1992.

80 Sevick EM, Jain RK. Viscous resistance to blood flow in solid tumors: effect of hematocrit on intratumor blood viscosity. Cancer Res 49:3513–3519, 1989.

81 Kluge M, Elger B, Engel T, Schaefer C, Seega J, Vaupel P. Acute effects of tumor necrosis factor α or lymphotoxin on global blood flow, laser doppler flux and bioenergetic status of subcutaneous rodent tumors. Cancer Res 52:2167–2173, 1992.

82 Bremner JCM, Counsell CJR, Adams GE et al. In vivo P nuclear magnetic resonance spectroscopy of experimental murine tumors and human tumour xenografts: effects of blood flow modification. Br J Cancer 64:862–866, 1991.

83 Larson SM. Radioimmunology. Imaging and therapy. Cancer 67:1253–1260, 1991.

84 Begent RH and Pedley RB. Antibody targeted therapy in cancer: comparison of murine and clinical studies. Cancer Treat Rev 17:373–378, 1990.

85 Shockley TR, Lin K, Nagy JA, Tompkins RG, Yarmush ML, Dvorak HF. Spatial distribution of tumor-specific monoclonal antibodies in human melanoma xenografts. Cancer Res 52:367–376, 1992.

86 Shockley TR, Lin K, Sung C et al. A quantitative analysis of tumor specific monoclonal antibody uptake by human melanoma xenografts: Effects of antibody immunological properties and tumor antigen expression levels. Cancer Res 52:357–366, 1992.

87 Sung C, Youle RJ, Dedrick RL. Pharmacokinetic analysis of immunotoxin uptake in solid tumors: role of plasma kinetics, capillary permeability and binding. Cancer Res 50:7382–7392, 1990.

88 Clark JW, Smith II JW, Steis RG et al. Interleukin 2 and lymphokine-activated killer cell continuous infusion interleukin 2 regimen. Cancer Res 50:7343–7350, 1990.

89 Griffith KD, Read EJ, Carrasquillo JS et al. In vivo distribution of adoptively transferred indium-lll-labeled tumor infiltrating lymphocytes (TIL) in patients with metastatic melanoma. J Natl Cancer Inst 8:1709–1717, 1989.

90 LeBerthon, Khawli LA, Alauddin M et al. Enhanced tumor uptake of macromolecules induced by a novel vasoactive interleukin-2 immunoconjugate. Cancer Res 51:2694–2698, 1991.

91 Schultz KR, Badger CC, Dombi GW, Greenberg PD, Bernstein ID. Effect of interleukin-2 n biodistribution of monoclonal antibody in tumor and normal tissues in mice bearing SL-2 thymoma. J Natl Cancer Inst 84:109–113, 1992.

92 Albelda SM, Daise M, Levine EM, Buck, CA. Identificati n and characterization of cell substratum adhesion receptors on cultured human endothelial cells. J Clin Invest 83:1992–2002, 1989.

93 Fujimori K, Covell DG, Fletcher JE, Weinstein JN. A modeling

analysis of monoclonal antibody percolation through tumors: A binding-site barrier. J Nucl Med 31:1191–1198, 1990.

94 Weinstein JN, van Osdol W. Early intervention in cancer using monoclonal antibodies and other biological ligands: microphar-macology and the 'binding site barrier'. Cancer Res 52:2747s-2751s, 1992.

95 Sung C, Shockley TR, Morrison PF, Dvorak HF, Yarmush ML, Dedrick RL. Predicted and observed effects of antibody affinity and antigen density on monoclonal antibody uptake in solid tumors. Cancer Res 52:377–384, 1992.

96 Goldenberg A, Masui H, Divgi C, Damrath H, Pentlow K, Mendelsohn J. Imaging of human tumor xenografts with an indium-III-labeled anti-epidermal growth factor receptor monoclonal antibody. J Natl Cancer Inst 81:1616–1625, 1989.

97 Philben VJ, Jakowatz JG, Beatty BG et al. The effect of tumor CEA content and tumor size on tissue uptake of indium III-labeled anti-CEA monoclonal antibody. Cancer 57:571–576, 1986.

98 Boerman O, Massuger L, Makkink K, Thomas C, Kenemans P, Poels L. Comparative in vitro binding characteristics and biodistribution in tumor-bearing athymic mice of anti-ovarian carcinoma monoclonal antibodies. Anticancer Res 10:1289–1296, 1990.

99 Presant CA, Wolf W, Albright MJ et al. Human tumor fluorouracil trapping: clinical correlations of in vivo ^{19}F nuclear magnetic resonance spectroscopy pharmacokinetics. J Clin Oncol 8:1868–1873, 1990.